Chemical speciation and mobilization of copper and zinc in naturally contaminated mine soils with citric and tartaric acids

Javier Pérez-Esteban , Consuelo Escolástico , Ana Moliner , Alberto Masaguer

HIGHLIGHTS

- Effects of citric and tartaric acid on metal mobilization in contaminated mine soils.
- Metal desorption was related to the type of organic acid and its concentration.
- Decreasing pH and dissolution of oxides by organic acids determined metal desorption.
- Low concentrations of organic acids did not increase metal mobilization.
- Citric acid at high concentrations (5–10 mM) remarkably promoted Cu mobilization.

ABSTRACT

A one-step extraction procedure and a leaching column experiment were performed to assess the effects of citric and tartaric acids on Cu and Zn mobilization in naturally contaminated mine soils to facilitate assisted phytoextraction. A speciation modeling of the soil solution and the metal fractionation of soils were performed to elucidate the chemical processes that affected metal desorption by organic acids. Different extracting solutions were prepared, all of which contained 0.01 M KNO_3 and different concentrations of organic acids: control without organic acids, 0.5 mM citric, 0.5 mM tartaric, 10 mM citric, 10 mM tartaric, and 5 mM citric +5 mM tartaric. The results of the extraction procedure showed that higher concentrations of organic acids increased metal desorption, and citric acid was more effective at facilitating metal desorption than tartaric acid. Metal desorption was mainly influenced by the decreasing pH and the dissolution of Fe and Mn oxides, not by the formation of soluble metal–organic complexes as was predicted by the speciation modeling. The results of the column study reported that low concentrations of organic acids did not significantly increase metal mobilization and that higher doses were also not able to mobilize Zn. However, 5–10 mM citric acid significantly promoted Cu mobilization (from 1 mg kg^{-1} in the control to 42 mg kg^{-1} with 10 mM citric acid) and reduced the exchangeable (from 21 to 3 mg kg^{-1}) and the Fe and Mn oxides (from 443 to 277 mg kg^{-1}) fractions. Citric acid could efficiently facilitate assisted phytoextraction techniques.

1. Introduction

Mining and smelting activities contribute significantly to metal contamination due to the discharge and dispersion of mine wastes into nearby agriculture soils, food crops and water ecosystems (Navarro et al., 2008). The accumulation of heavy metals in soil can negatively impact the health of humans and animals, plant growth and soil microbial activity (Vamerali et al., 2010).

A promising strategy for remediating metal-contaminated sites involves phytoextraction, an *in situ* technique in which plants are used to remove pollutants from the environment (Vamerali et al., 2010). However, the slow desorption of metals in soils has been a major limitation for successful phytoextraction. The bioavailability of metals is affected by several soil factors, such as pH, cation exchange capacity, organic matter content, the speciation of the metal and the metal itself (Evangelou et al., 2007).

Phytoextraction can be improved by the addition of appropriate amendments or chelating agents to the soil to increase metal mobility and enhance its uptake by plants. Various synthetic chelating agents have been used in assisted phytoextraction studies. One of them is ethylenediaminetetraacetic acid (EDTA), which is

probably the most efficient chelating agent for enhancing metal uptake by plants (Blaylock et al., 1997). However, the slow degradation rate and the long persistence of EDTA in the soil could lead to an increased risk of groundwater pollution due to the leaching of metals; moreover, this compound could be toxic for plants and microorganisms (Evangelou et al., 2007).

Several compounds have been proposed as alternatives to EDTA and other synthetic chelating agents. These proposed compounds should be a compromise between their fast degradation and their ability to efficiently enhance phytoextraction. Among them, low-molecular-weight organic acids (LMWOAs) are natural compounds that originate from root exudates, microbial metabolites and the decomposition of soil organic matter (Jones, 1998). These easily biodegradable carboxylic acids, such as citric and tartaric acids, reduce soil pH and are capable of forming soluble complexes, thereby playing an important role in metal mobility and in the subsequent accumulation of metals in plants (Evangelou et al., 2007). However, several studies have observed low extraction efficiencies when these compounds have been applied at low doses because of their rapid biodegradation and their sorption onto soil particles (Evangelou et al., 2006, 2008; Liu et al., 2008). Although the effects of LMWOAs on metal desorption from soils with induced contamination have been widely studied (Qin et al., 2004; Evangelou et al., 2006, 2008; Nascimento, 2006; Schwab et al., 2008), there is limited information on the desorption behavior of metals from naturally contaminated soils with long aging times.

The aims of this study were to assess and compare through a one-step extraction procedure and a soil column experiment the effects of two natural LMWOAs, citric and tartaric acids, which are commonly present in soils, on Cu and Zn mobilization in naturally contaminated mine soils to facilitate future assisted phytoextraction techniques. This study also attempted to elucidate the main chemical processes that affect metal desorption by LMWOAs in these soils by means of speciation modeling using Visual Minteq and metal fractionation.

2. Material and methods

2.1. Soil characteristics

Two heavy metal-contaminated soils from the north of Madrid (Spain) were selected for this study. The first site was situated at the village Garganta de los Montes (G), which is close to a copper mine that was abandoned in 1965. The second site was situated in El Cuadron (C), where an old blende mine that was abandoned in 1862 is located. The soils at these site locations have been classified as humic and dystric cambisols by the FAO (Pastor et al., 2007).

Samples were collected at these sites from the top 20 cm of the soil and were air-dried and sieved to <2 mm for analysis. Soil samples were analyzed for oxidizable organic carbon (OXC) using the modified Walkley–Black procedure that was described by Nelson and Sommers (1996). The electrical conductivity (EC) and pH were analyzed in deionized water extracts (1:2.5 w/w). The cation exchange capacity (CEC) was determined using the barium chloride method adjusted to soil pH (Rhoades, 1982). Total Fe and Mn oxides were determined by performing three sequential extractions using an ascorbate-oxalate solution at 90–95 °C (Shuman, 1982). Amorphous Fe and Mn oxides were extracted using ammonium oxalate in the dark (Smith and Mitchell, 1987). The texture was determined using the Bouyoucos hydrometer method (Day, 1965). The point of zero net charge (PZNC) was measured using a KCl saturation method, which was previously described by Zelazny et al. (1996).

The total heavy metal content (Cu and Zn) in the soil samples was determined using microwave-assisted acid digestion with *aqua regia* (ISO 11466, 1995).

The metal concentrations in the soil extracts were determined by atomic absorption spectrophotometry (AAS) using a Perkin Elmer AAnalyst 400. Measurements were carried out in an air/acetylene flame (2 L min^{-1} of acetylene and 17 L min^{-1} of air). Single-element hollow cathode lamps were used at the corresponding wavelength (Cu: 324.8 nm; Zn: 213.9 nm; Fe: 248.3 nm; Mn: 279.5 nm) and slit width (Cu and Zn: 0.7 nm; Fe and Mn: 0.2 nm). Copper and Zn concentrations were directly measured in the extracts, whereas Fe and Mn were measured in a 0.2% calcium chloride solution to eliminate interferences. The element standard solutions used for calibration were prepared by suitable dilution of stock standard of 1000 mg L^{-1} with the same extracting solutions used for the samples and blanks.

All analyses were performed in triplicate, and the values were adjusted for oven-dried (overnight at 105 °C) soil.

The characterization of the two types of soils is shown in Table 1. Both were slightly acidic, loamy sand and poor in organic matter. They also contained a large amount of Fe oxides and had low salinity and cation exchange capacity (CEC). Both soils possessed total concentrations of Cu that exceeded the European Union maximum permitted levels for agricultural soils that receive sewage sludge at pH 6–7 (50–140 mg kg^{-1}; Council of the European Communities, 1986).

2.2. Total LMWOAs-extractable metals

To determine the total amount of metals that were extractable by LMWOAs, a one-step extraction procedure was performed on both soils as follows. First, a total of 5 g of soil samples was shaken for 48 h with 1 L of a solution that contained a background electrolyte (0.01 M KNO_3) and different concentrations of citric and tartaric acids. Six different treatments were prepared for each soil, including without any organic acids (Control, pH 5.7), 0.5 mM citric acid (C0.5, pH 3.3), 0.5 mM tartaric acid (T0.5, pH 3.3), 10 mM citric acid (C10, pH 2.4), 10 mM tartaric acid (T10, pH 2.4), and 5 mM citric acid +5 mM tartaric acid (CT5, pH 2.4). Soil extracts were then filtered and analyzed for pH and metal concentrations (Cu and Zn). This experiment was performed in triplicate, and the values were adjusted for oven-dried soil.

Table 1
Properties of the mine soils.

Characteristic[a]	*G*	*C*
Clay (%)	3.1	3.3
Silt (%)	18.8	17.3
Sand (%)	78.2	79.4
pH (1:2.5 w/w)	6.2	5.5
EC (dS m^{-1})	0.08	0.10
CEC ($cmol_c$ kg^{-1})	4.74	4.79
OXC (%)	0.74	1.37
PZNC	4.5	2.6
Total Fe oxides (g Fe kg^{-1})	11.9	7.31
Amorph. Fe ox. (g Fe kg^{-1})	2.04	1.11
Total Mn oxides (g Mn kg^{-1})	0.26	0.28
Amorph. Mn ox. (g Mn kg^{-1})	0.16	0.23
Total Cu (mg kg^{-1})[b]	913	248
Total Zn (mg kg^{-1})	203	146

[a] Texture (Day, 1965); pH, EC: electrical conductivity (1:2.5 w/w); CEC: cation exchange capacity (Rhoades, 1982); OXC: oxidizable organic carbon (Nelson and Sommers, 1996); PZNC: point of zero net charge (Zelazny et al., 1996); Total Fe and Mn oxides (Shuman, 1982); Amorphous Fe and Mn oxides (Smith and Mitchell, 1987); Total Cu and Zn (ISO 11466, 1995).

[b] European Union limits (mg kg^{-1}) for agricultural soils receiving sewage sludge (pH 6–7): Cu 50–140, Zn 150–300 (Council of the European Communities, 1986).

2.3. Chemical speciation modeling of the soil solution

The speciation of metals in the soil solution was simulated using the computer program Visual Minteq v.3.0 (Gustafsson, 2011) to predict the formation of complexes between metals and the organic acids that were added during the experiment as described in Section 2.2. Data were calculated using the equilibrium constants for aqueous complexes from the default Visual Minteq database (Table 2). The measured values of pH and metal concentrations in soil extracts, and the organic acids and background electrolyte concentrations of the solutions applied were used as the input data. Data sets were calculated considering the solution in equilibrium with atmospheric CO_2 (3.8×10^{-4} atm). The Davies method for activity correction was selected, and oversaturated solids were not allowed to precipitate. No surface complexation models were considered in this study.

2.4. Leaching column experiment

A leaching column experiment was performed to evaluate the effect of LMWOAs on metal mobilization. Glass columns of 6 cm diameter and 15 cm length were used. The base of each column was covered with a nylon mesh to retain the soil, and a plastic tube was connected to collect the leachate. The columns were filled with 300 g of soil and were initially irrigated with 150 mL of deionized water for 48 h prior to the start of the experiment to humidify and homogenize the soil. Pretreatment with deionized water was not needed in the one-step extraction procedure (Section 2.2) due to the high solution:ratio and shaking time applied in that experiment. Each column was manually irrigated every three days with 50 mL of one of the solutions described in Section 2.2, and the leachates were collected in plastic flasks and analyzed for pH and Cu and Zn concentrations. A total of 27 sequential extractions were performed (79 days), and the experiment was performed in triplicate.

2.5. Balance and fractionation of metals after the leaching column experiment

Soils from the column experiment were air-dried and analyzed for pH and metal concentrations in the different soil fractions. The leached and the original un-leached soils were analyzed to study the changes in metal fractionation following the column experiment with LMWOAs. The metal fractionation of the soil samples was performed following the sequential extraction procedure of Tessier et al. (1979) with the exception of the residual fraction, which was extracted using an HNO_3–HCl acid digestion. This procedure is designed to separate metals into six operationally defined fractions: water-soluble, exchangeable, acid-soluble, bound to Fe–Mn oxides, bound to organic matter and sulfides, and residual.

All reagents used were of analytical grade or better. Double deionized water was used for all dilutions, and all plastic and glassware were soaked in a 5% HNO_3 solution overnight and rinsed with distilled water before use.

Statistical treatments of the experimental data were performed using SPSS (Statistical Package for the Social Sciences) 17.0 (SPSS, Inc.) software. Mean values were compared through a one-way ANOVA using Tukey's test ($P < 0.05$). Standard errors (SEs) were calculated to determine the variability of the means between replicates.

3. Results and discussion

3.1. Total LMWOAs-extractable metals

Table 3 shows the total concentrations of Cu and Zn that were extracted by LMWOAs and the pH of the soil extracts in the one-step extraction procedure.

The presence of LMWOAs significantly increased the metal desorption in both soils. The desorption behavior of Cu was related to the specific organic acids and their concentrations. These organic acids were able to extract up to 40% of the potentially available Cu concentration (sum of the first five fractions: 778 mg kg^{-1}) in treatment C10 of soil G and up to 56% in C10 of soil C (170 mg kg^{-1}) (see untreated soil in Table 4). They were also able to extract up to 56% of the potentially available Zn concentration (68 mg kg^{-1}) in C10 of soil G and up to 74% in C10 of soil C (70 mg kg^{-1}) (Table 5).

In the control treatments in which no LMWOAs were added, the Cu concentrations were significantly lower than in the other treatments in the presence of the organic acids ($P < 0.05$). The copper concentrations in the soil extracts were also significantly higher

Table 2
Solubility constants and heats of reaction used to calculate the species distribution in soil solution.

Reaction	log*K* (25 °C)	ΔH_r (kJ mol^{-1})
$Cu^{2+} + CO_3^{2-} \leftrightarrow CuCO_3$	6.77[a]	0[a]
$Cu^{2+} + CO_3^{2-} + H^+ \leftrightarrow CuHCO$	12.129[a]	0[a]
$Cu^{2+} + NO_3^- \leftrightarrow CuNO_3^+$	0.5[a]	−4.1[a]
$Cu^{2+} + Citrate^{3-} \leftrightarrow CuCitrate^-$	7.57[b]	0[b]
$Cu^{2+} + Citrate^{3-} + H^+ \leftrightarrow CuHCitrate$	11.02[c]	11.3[c]
$Cu^{2+} + Citrate^{3-} + 2H^+ \leftrightarrow CuH_2Citrate^+$	13.23[b]	0[b]
$Cu^{2+} + Tartrate^{2-} \leftrightarrow CuTartrate$	3.97[c]	0[c]
$Cu^{2+} + Tartrate^{2-} + H^+ \leftrightarrow CuHTartrate^+$	6.29[c]	0[c]
$Zn^{2+} + CO_3^{2-} \leftrightarrow ZnCO_3$	4.76[a]	0[a]
$Zn^{2+} + CO_3^{2-} + H^+ \leftrightarrow ZnHCO_3^+$	11.829[a]	0[a]
$Zn^{2+} + NO_3^- \leftrightarrow ZnNO_3^+$	0.4[a]	−4.6[a]
$Zn^{2+} + Citrate^{3-} \leftrightarrow ZnCitrate^-$	6.06[c]	8[c]
$Zn^{2+} + Citrate^{3-} + H^+ \leftrightarrow ZnHCitrate$	10.24[c]	0[c]
$Zn^{2+} + Tartrate^{2-} \leftrightarrow ZnTartrate$	3.43[c]	0[c]
$Zn^{2+} + Tartrate^{2-} + H^+ \leftrightarrow ZnHTartrate^+$	5.91[c]	0[c]
$K^+ + NO_3^- \leftrightarrow KNO_3$	−0.19[a]	−12[a]
$K^+ + Citrate^{3-} \leftrightarrow KCitrate^{2-}$	1.1[c]	5.4[c]
$K^+ + Tartrate^{2-} \leftrightarrow KTartrate^-$	0.83[c]	0[c]

[a] Smith et al. (2003).
[b] IUPAC (1994).
[c] Smith et al. (2001).

Table 3
Total LMWOAs-extractable Cu and Zn concentrations (mg kg^{-1}) and pH values using different concentrations of LMWOAs and a background electrolyte in a one-step extraction procedure.

Treatment	pH	Cu	Zn
Soil G			
Control	5.9 ± 0.1	4.1a ± 0.5	5.6a ± 0.2
C0.5	3.5 ± 0.0	171b ± 1.6	23.9c ± 0.3
T0.5	3.4 ± 0.0	143b ± 3.4	19.2b ± 0.3
C10	2.5 ± 0.0	308d ± 7.3	37.6e ± 0.7
T10	2.4 ± 0.0	235c ± 16	32.9d ± 0.3
CT5	2.5 ± 0.0	307d ± 6.8	37.5e ± 0.8
Soil C			
Control	5.7 ± 0.0	4.2a ± 0.2	15.1a ± 0.2
C0.5	3.5 ± 0.0	42.9b ± 0.1	39.4b ± 0.7
T0.5	3.4 ± 0.0	39.7b ± 0.8	39.5b ± 0.6
C10	2.5 ± 0.0	94.3d ± 1.4	52.1c ± 2.5
T10	2.4 ± 0.0	92.3d ± 2.8	50.0c ± 0.5
CT5	2.5 ± 0.0	84.5c ± 0.8	49.9c ± 0.5

Control: background electrolyte (0.01 M KNO_3); C0.5: 0.5 mM citric acid + background electrolyte; T0.5: 0.5 mM tartaric acid + background electrolyte; C10: 10 mM citric acid + background electrolyte; T10: 10 mM tartaric acid + background electrolyte; CT5: 5 mM citric acid + 5 mM tartaric acid + background electrolyte.
Mean ± SE, $n = 3$.
Values within a column followed by the same letter are not significantly different among treatments of the same soil ($P < 0.05$).

Table 4
Concentrations of Cu (mg kg^{-1}) in the different fractions obtained by a sequential extraction procedure (Tessier et al., 1979) and pH values of soils at the beginning (untreated) and at the end of the leaching column experiment using different concentrations of LMWOAs and a background electrolyte.

Treatment	pH	Water	Exch.	Acid-sol	Fe-Mn ox.	Organic-S	Residual	Sum
Soil G								
Untreated	6.2a ± 0.0	1.5abc ± 0.1	21d ± 0.5	89a ± 2.8	443b ± 21	223a ± 23	175a ± 17	952b ± 51
Control	6.5b ± 0.0	2.0abc ± 0.3	14c ± 0.6	89a ± 1.0	309a ± 4.0	316b ± 6.8	169a ± 0.8	894ab ± 2.2
C0.5	6.6bc ± 0.0	2.2c ± 0.1	12bc ± 0.2	88a ± 1.9	303a ± 5.6	266ab ± 8.0	141a ± 2.4	811ab ± 18
T0.5	6.8 cd ± 0.0	1.7abc ± 0.0	9.7b ± 0.4	85a ± 1.5	295a ± 4.7	260ab ± 3.1	145a ± 2.5	798ab ± 1.0
C10	7.3e ± 0.1	2.2bc ± 0.2	3.3a ± 0.5	88a ± 2.1	277a ± 1.8	253ab ± 8.9	139a ± 8.0	763a ± 18
T10	6.5b ± 0.0	1.3a ± 0.0	9.1b ± 1.1	98a ± 1.1	315a ± 0.7	271ab ± 8.2	151a ± 1.4	847ab ± 8.8
CT5	6.9d ± 0.1	1.4ab ± 0.1	4.1a ± 0.6	91a ± 3.6	329a ± 7.2	299ab ± 2.1	180a ± 6.2	900ab ± 11
Soil C								
Untreated	5.5a ± 0.0	2.8ab ± 0.2	13d ± 0.5	35a ± 1.3	69b ± 1.0	51a ± 3.3	71a ± 2.3	240b ± 7.0
Control	5.9ab ± 0.1	2.7a ± 0.1	5.5bc ± 0.8	30a ± 0.4	55a ± 1.4	53a ± 1.2	71a ± 0.6	216ab ± 2.6
C0.5	6.0b ± 0.0	3.6ab ± 0.1	5.9bc±0.1	31a ± 0.2	54a ± 0.6	53a ± 0.5	69a ± 1.9	214a ± 0.1
T0.5	5.9ab ± 0.0	3.8ab ± 0.0	7.8c ± 0.1	35a ± 1.4	52a ± 1.1	54a ± 0.2	69a ± 3.4	221ab ± 6.2
C10	6.2bc ± 0.1	3.6ab ± 0.6	3.3ab ± 0.1	31a ± 0.2	53a ± 0.6	53a ± 1.8	64a ± 2.1	208a ± 0.8
T10	5.9ab ± 0.1	3.4ab ± 0.2	6.3c ± 0.0	35a ± 0.3	52a ± 0.2	54a ± 0.4	70a ± 0.9	223ab ± 0.5
CT5	6.4c ± 0.1	4.0b ± 0.1	1.5a ± 0.1	32a ± 0.2	53a ± 1.1	52a ± 2.1	69a ± 1.3	212a ± 4.3

Untreated: soil at the beginning of the column experiment; Control: background electrolyte (0.01 M KNO_3); C0.5: 0.5 mM citric acid + background electrolyte; T0.5: 0.5 mM tartaric acid + background electrolyte; C10: 10 mM citric acid + background electrolyte; T10: 10 mM tartaric acid + background electrolyte; CT5: 5 mM citric acid + 5 mM tartaric acid + background electrolyte.
Mean ± SE, n = 3.
Values within a column followed by the same letter are not significantly different among treatments of the same soil ($P < 0.05$).

Table 5
Concentrations of Zn (mg kg^{-1}) in the different fractions obtained by a sequential extraction procedure (Tessier et al., 1979) at the beginning (untreated) and at the end of the leaching column experiment using different concentrations of LMWOAs and a background electrolyte.

Treatment	Water	Exch.	Acid-sol	Fe-Mn ox.	Organic-S	Residual	Sum
Soil G							
Untreated	0.4b ± 0.0	5.5b ± 0.2	3.8a ± 0.1	48d ± 0.9	10a ± 0.4	131a ± 1.0	200a ± 0.2
Control	0.4b ± 0.0	6.0b ± 0.4	4.1a ± 0.1	35a ± 0.9	14bc ± 0.5	141a ± 1.5	203a ± 1.5
C0.5	0.4b ± 0.0	5.3b ± 0.2	4.2a ± 0.1	34a ± 0.4	12ab ± 0.4	141a ± 3.9	197a ± 4.3
T0.5	0.3b ± 0.0	5.6b ± 0.0	4.3a ± 0.0	37abc ± 0.0	15c ± 0.2	136a ± 0.3	198a ± 0.0
C10	0.3b ± 0.0	2.7a ± 0.5	6.3b ± 0.2	36ab ± 0.5	13bc ± 0.1	140a ± 2.0	199a ± 1.9
T10	0.1a ± 0.0	5.3b ± 0.2	5.7b ± 0.2	39bc ± 0.4	14bc ± 0.5	138a ± 3.2	203a ± 4.1
CT5	0.1a ± 0.0	3.2a ± 0.7	6.7b ± 0.6	41c ± 0.1	14bc ± 0.4	141a ± 0.4	208a ± 2.0
Soil C							
Untreated	1.4b ± 0.1	26c ± 1.0	5.8a ± 0.2	29b ± 0.5	8.1b ± 0.2	57a ± 2.4	127a ± 4.2
Control	0.5a ± 0.0	23bc ± 0.5	7.0abc ± 0.4	24a ± 0.3	1.5a ± 0.1	65a ± 1.1	121a ± 1.0
C0.5	0.5a ± 0.1	23bc ± 0.4	6.5ab ± 0.1	23a ± 0.5	0.6a ± 0.1	61a ± 0.1	115a ± 0.9
T0.5	0.6a ± 0.2	24bc ± 0.7	8.2bc ± 0.0	23a ± 0.7	1.9a ± 0.4	62a ± 3.4	119a ± 5.6
C10	0.4a ± 0.0	19ab ± 2.1	9.2c ± 0.3	27b ± 0.3	1.5a ± 0.1	61a ± 0.0	117a ± 0.2
T10	0.5a ± 0.0	22bc ± 1.7	7.7abc ± 0.2	24a ± 0.1	2.1a ± 0.2	64a ± 0.4	121a ± 1.9
CT5	0.4a ± 0.1	16a ± 0.4	12d ± 1.1	28b ± 1.2	1.9a ± 0.7	64a ± 1.5	123a ± 3.9

Untreated: soil at the beginning of the column experiment; Control: background electrolyte (KNO_3 0.01 M); C0.5: citric acid 0.5 mM + background electrolyte; T0.5: tartaric acid 0.5 mM + background electrolyte; C10: citric acid 10 mM + background electrolyte; T10: tartaric acid 10 mM + background electrolyte; CT5: citric acid 5 mM + tartaric acid 5 mM + background electrolyte.
Mean ± SE, $n = 3$.
Values within a column followed by the same letter are not significantly different among treatments of the same soil ($P < 0.05$).

with increasing LMWOAs concentrations (10 mM) in the extraction solutions ($P < 0.05$).

In soil G, the addition of a high concentration of citric acid (C10) resulted in a significantly higher Cu desorption than tartaric acid that was applied at the same concentration (T10) ($P < 0.05$), showing that citric acid facilitated a greater capacity for Cu to mobilize. The Cu concentration in the mixture of citric and tartaric acid at 5 mM (CT5) was similar to that obtained in C10 and was significantly higher than in T10 ($P < 0.05$). Similar results were obtained by Gao et al. (2003) when different concentrations of citric and tartaric acid were added to soil, indicating that LMWOAs that were applied in high concentrations (>2 mM for citrate and >15 mM for tartrate) enhanced Cu desorption. Additionally, others (Gao et al., 2003; Qin et al., 2004) reported that citric acid was more effective for Cu desorption than other LMWOAs (e.g., tartaric, malic, and acetic acids).

In soil C, no significant differences in Cu concentration were found between citric and tartaric acid treatments that were applied at the same concentration. The copper concentration in treatment CT5 represented an intermediate level.

The zinc concentrations in the soil extracts containing LMWOAs followed the same trend as Cu. There were significantly lower Zn concentrations ($P < 0.05$) in the control treatments. The application of LMWOAs at high concentrations (10 mM) extracted significantly higher Zn concentrations than at low concentration (0.5 mM) ($P < 0.05$). In soil G, citric acid treatments (C0.5 and C10) extracted a greater amount of Zn in comparison to tartaric treatments (T0.5 and T10), whereas there were no significant differences between citric and tartaric treatments in soil C. Schwab et al. (2008) also reported higher Zn mobilization in soils in the presence of citric acid compared to other LMWOAs.

The pH of the soil extracts in the control samples were 5.7–5.9 (Table 3), whereas the extracts of the LMWOAs treatments reached pH values of 2.4–3.5. Therefore, it must be considered to compare results that different desorption mechanisms were involved in the control treatments and those with LMWOAs. However, the

comparison between these treatments could be useful for showing the effectiveness of both LMWOAs in metal desorption.

In the control treatments, where only KNO_3 was added, an ion exchange and protonation of the mineral surfaces were the main processes that controlled metal desorption. Conversely, in the LMWOAs treatments the metal desorption was probably governed by the low pH that was reached in the soil extracts, which reduced the negative surface charge of the soil particles, the formation of soluble metal–organic chelates and the dissolution of Fe and Mn oxides by organic acids, thereby mobilizing their associated metals. Johnson and Loeppert (2006) studied the effectiveness of organic acids in the dissolution of Fe oxides, reporting that citric and tartaric acid were able to release a considerable amount of Fe from oxides, especially at low pH values, and that the dissolution was greater for amorphous oxides than for crystalline oxides. These authors also observed that the effectiveness was higher for citric acid than for tartaric acid. Schwab et al. (2008) reported that increased concentrations of citric acid were associated with higher effluent Fe in a soil column study. The different effectiveness in the dissolution of metal oxides between these LMWOAs could explain the lack of significant differences in the concentrations of metals between citric and tartaric treatments in soil C (Table 3), which possessed a lower Fe oxides content than soil G (Table 1). Therefore, in soil C a low amount of metals was released from these compounds using citric acid, whereas in soil G this organic acid managed to release a significantly greater amount of metals in comparison to tartaric acid.

3.2. Chemical speciation modeling of the soil solution

The higher metal desorption in the citric acid treatments described in Section 3.1 could have been due to the fact that citric acid is a tricarboxylic acid, which facilitates greater complexation ability than tartaric acid, a dicarboxylic acid (Jones, 1998). Thus, the stability constants ($\log K$) of the chelates that are formed between citric acid and these metals (5.9 for Cu and 5.0 for Zn; Barton and Abadía, 2006) are higher than those with tartaric acid (3.2 for Cu and 2.7 for Zn; Martell and Smith, 1976–1989).

Table 6 shows the predicted proportions of the metal–organic complexes with citrate and tartrate and other chemical species of Cu and Zn in the soil solution with respect to the total extracted metal concentrations, which were simulated using Visual Minteq and the data that were described in Table 3. The degradation of the LMWOAs was not considered in the simulation.

Free Cu^{2+} was generally the predominant species in the soil solutions, except for the control treatment of soil G in which the inorganic complexes were the most abundant species and in C0.5 of both soils. The concentrations of Cu that were complexed with the anion of the background electrolyte, $Cu(NO_3)_2^0$ and $CuNO_3^+$, were insignificant in all of the treatments, suggesting that the exchange with the cation K^+ of the background electrolyte was the main process that was involved with Cu desorption in the control treatment.

The proportions of Cu that were complexed with organic ligands in the treatments with high concentration of LMWOAs (C10, T10, and CT5) were lower or similar to those in C0.5 and T0.5. Despite the higher amounts of organic acids that were added, the low pH of the soil solutions in C10, T10, and CT5 (Table 3) reduced the chelating ability of the LMWOAs. At these pH values, the predominant organic ligands of citric acid (pK_as: 3.13, 4.78, 6.43; Serjeant and Dempsey, 1979) and tartaric acid (pK_as: 3.03, 4.46; Serjeant and Dempsey, 1979) were H_3-citrate^0 and H_2-tartrate^0, which possessed a neutral charge and no chelating ability. At the pH values found in C0.5 and T0.5 (Table 3), the most abundant organic ligands were H_2-citrate$^-$ and H-tartrate$^-$, which were able to form complexes with metals.

Free Zn^{2+} was the predominant species in soil solution in all of the treatments. The concentrations of other soluble Zn species were very low. The lower affinity of Zn for organic ligands in comparison to Cu and the low pH that was reached in the soil solutions did not allow the formation of a significant amount of stable complexes with Zn.

These results show that the low concentration of metal–organic complexes predicted in soil solution did not explain the significant amounts of metals that were extracted by the LMWOAs due to the low pH reached in the extracts. The main processes that affected Cu and Zn desorption in the one-step extraction experiment could have been the acidification and the dissolution of Fe and Mn oxides by organic acids.

3.3. Leaching column experiment

Columns were used to study the mobilization of metals in the presence of organic acids under conditions that more closely simulated field conditions than those in the one-step extraction procedure. Table 7 shows the pH values of the last leachate and the accumulated Cu and Zn concentrations in the leachates from the soil columns, which were calculated as the sum of the metal concentrations during the 27 extractions.

Table 6
Predicted percentages of the different chemical species of Cu and Zn in soil solution with respect to the total extracted Cu and Zn concentrations in the one-step extraction procedure using different concentrations of LMWOAs and a background electrolyte simulated with Visual Minteq.

Treatment	Free Cu^{2+}	Inorg. Cu complexes	Cu-citrate complexes	Cu-tartrate complexes	Free Zn^{2+}	Inorg. Zn complexes	Zn-citrate complexes	Zn-tartrate complexes
Soil G								
Control	33	77	0	0	75	25	0	0
C0.5	46	1	53	0	89	3	8	0
T0.5	79	2	0	19	93	0	0	7
C10	61	1	38	0	93	2	5	0
T10	78	1	0	21	90	2	0	8
CT5	71	2	16	11	91	2	2	5
Soil C								
Control	86	14	0	0	100	0	0	0
C0.5	46	2	52	0	90	0	10	0
T0.5	78	3	0	19	92	1	0	7
C10	61	1	38	0	93	1	6	0
T10	77	1	0	22	88	3	0	9
CT5	71	1	17	11	92	0	3	5

Control: background electrolyte (0.01 M KNO_3); C0.5: 0.5 mM citric acid + background electrolyte; T0.5: 0.5 mM tartaric acid + background electrolyte; C10: 10 mM citric acid + background electrolyte; T10: 10 mM tartaric acid + background electrolyte; CT5: 5 mM citric acid + 5 mM tartaric acid + background electrolyte.

Table 7
Accumulated Cu and Zn concentrations (mg kg^{-1}) in the 27 leachates and pH values of the last leachate from soil columns using different concentrations of LMWOAs and a background electrolyte.

Treatment	pH	Cu	Zn
Soil G			
Control	6.1 ± 0.0	1.1a ± 0.0	2.3 cd ± 0.0
C0.5	6.5 ± 0.1	1.1a ± 0.1	2.3bcd ± 0.0
T0.5	6.5 ± 0.1	0.9a ± 0.0	1.7a ± 0.0
C10	3.7 ± 0.2	42c ± 2.4	2.9d ± 0.2
T10	3.0 ± 0.3	10ab ± 0.6	1.8ab ± 0.0
CT5	8.6 ± 0.1	22b ± 7.8	1.9abc ± 0.1
Soil C			
Control	5.3 ± 0.3	2.0a ± 0.2	8.8a ± 1.2
C0.5	4.9 ± 0.4	2.2a ± 0.0	11a ± 0.5
T0.5	5.9 ± 0.7	1.9a ± ± 0.1	9.1a ± 0.4
C10	3.8 ± 0.3	11c ± 1.9	6.6a ± 0.2
T10	3.5 ± 0.5	5.2ab ± 0.6	10a ± 0.4
CT5	8.1 ± 0.1	6.9b ± 0.0	6.1a ± 1.8

Control: background electrolyte (0.01 M KNO_3); C0.5: 0.5 mM citric acid + background electrolyte; T0.5: 0.5 mM tartaric acid + background electrolyte; C10: 10 mM citric acid + background electrolyte; T10: 10 mM tartaric acid + background electrolyte; CT5: 5 mM citric acid + 5 mM tartaric acid + background electrolyte.
Mean ± SE, $n = 3$.
Values within a column followed by the same letter are not significantly different among treatments of the same soil ($P < 0.05$).

The effects caused by the LMWOAs on Cu desorption were similar in both soils. There were no significant differences in the Cu concentration between the control treatment and treatments C0.5 and T0.5. In these treatments, the lower concentrations of added LMWOAs and the higher pH values were insufficient to extract a significant amount of Cu. The formed Cu metal–organic complexes with positive charges could have been sorbed by soil particles that contain negative surface charges at pH values above the PZNC (Table 1), thereby reducing Cu desorption. The fast degradation of the small amount of LMWOAs that were applied to the soil must also be considered (Evangelou et al., 2007, 2008). Gao et al. (2003) showed similar results, reporting that Cu desorption was inhibited in the presence of low concentrations of citric (<0.1 mM) and tartaric acid (<0.5 mM), most likely due to the sorption of the organic ligands and their bounded metals to the soil particles.

Additionally, treatment T10 with 10 mM tartaric acid did not significantly increase Cu concentration in the leachates. However, the treatments with high concentrations of citric acid (C10 and CT5) managed to extract a significantly higher amount of Cu in the same way as the one-step extraction experiment. When high concentrations of LMWOAs are added to soil, the proportion of organic ligands in the soil solutions with respect to the organic ligands that are sorbed by soil becomes larger, enhancing Cu desorption (Gao et al., 2003). Liu et al. (2008) suggested that the addition of LMWOAs should be greater than 10 mM kg^{-1} to result in significant effects on metal solubility (the dosage of citric acid applied in treatment C10 was 45 mM kg^{-1} of soil). Evangelou et al. (2006) reported that the amount of Cu that was extracted from soil columns using citric acid was significantly higher than the Cu amounts that were extracted by EDTA.

The organic acids did not significantly affect Zn desorption in this column study in either soils (Table 7). There were no significant differences between the different treatments in soil C, and in soil G, there were no significant differences between the control and the treatments with citric acid (C0.5, C10, and CT5). Even the addition of tartaric acid (T0.5 and T10) slightly reduced the Zn concentrations in the leachates of soil G. Different results were obtained by other authors (Nascimento, 2006; Schwab et al., 2008; Vesely et al., 2012), who reported that the application of citric, tartaric and other organic acids at high concentrations desorbed substantial amounts of Zn from soil.

In this column experiment it must also be considered to compare results that different desorption mechanisms were involved in the control treatments and the LMWOAs treatments. The treatments with low doses of LMWOAs (C0.5 and T0.5) presented similar pH values and metal concentrations (Table 7) to those in the control treatments. Metal desorption was mainly affected by its exchange with the cation K^+ of the background electrolyte and by the protonation of mineral surfaces in these cases. In the treatments with high doses of LMWOAs (C10 and T10), Cu desorption was governed by the low pH, the dissolution of Fe and Mn oxides and the formation of complexes between organic ligands and Cu. The higher amount of Cu extracted by citric acid could be explained by the greater effectiveness in oxides dissolution and the stronger chelating ability of this organic acid with Cu in comparison to tartaric acid. Zinc desorption was not so determined as Cu by the type and concentration of organic acids due to the weaker complexation ability of the organic ligands with Zn. In treatments CT5, which exhibited an intermediate Cu mobilization ability, metal desorption was probably also dominated by the dissolution of oxides and the complexation of metals, but the pH of the extracts was considerably higher (Table 7). Evangelou et al. (2006) also observed an increase in the pH of the leachates after a column experiment using LMWOAs. This was probably due to the biodegradation of the LMWOAs, which resulted in a consumption of H^+ and the liberation of OH^- (Evangelou et al., 2006, 2008). Maybe the greater variety of organic substrates for microorganisms in treatments CT5 increased the biodegradation activity and the pH of the leachates. The desorption of cations by the LMWOAs and the reducing conditions in the columns could also have led to the consumption of H^+ in these treatments.

In our experiments, the amounts of Cu and Zn that were extracted by LMWOAs in the column study were markedly lower than the metals that were extracted in the one-step extraction experiment. These lower metal concentrations were due to the higher pH values reached in the extracts and the lower solution:soil ratio and reaction time applied in each extraction in the column study in comparison to the one-step extraction experiment.

3.4. Balance and fractionation of metals after the leaching column experiment

Both soils were analyzed for metal fractionation after the column experiment and were compared with the untreated soils to study the fractions of metals that were more likely to be extracted by LMWOAs. Table 4 shows the pH values and Cu concentrations in the different soil fractions at the beginning and at the end of the column experiment using LMWOAs.

In most cases, the pH values of the soils significantly increased after the column study ($P < 0.05$). The biodegradation of the organic acids during the experiment most likely increased the soil pH (Evangelou et al., 2006), and the reducing conditions in the soil columns led to the reductive dissolution of Fe and Mn oxides, which consume protons (Charlatchka and Cambier, 2000).

There were no significant differences in water-soluble Cu and in acid-soluble Cu between the untreated soil and the soil at the end of the experiment. However, exchangeable Cu significantly decreased after the column study in all of the treatments but especially in those with a high concentration of citric acid (C10 and CT5) ($P < 0.05$).

There was also a significant decrease in the Cu that was bounded to Fe and Mn oxides in all of the treatments at the end of the experiment ($P < 0.05$). This decrease was most likely due to the dissolution of these oxides by the LMWOAs and the reducing

conditions in the columns, which enhanced the mobilities of the metals that were bounded to them (Charlatchka and Cambier, 2000). However, the Cu concentration of the bound-to-organic-matter-and-sulfides fraction significantly increased after the control treatment of soil G ($P < 0.05$). The reducing conditions in the columns could also have led to the formation of insoluble sulfides with this metal.

In soil G, the sums of all Cu fractions significantly decreased after the experiment when citric acid was added at 10 mM (C10), while in soil C, these sums significantly decreased in C0.5, C10, and CT5 ($P < 0.05$). Thus, citric acid appeared to be an effective Cu-extracting agent in these soils.

The Zn concentrations in the different fractions at the beginning and at the end of the column experiment are shown in Table 5. The addition of high concentrations of citric acid (C10 and CT5) significantly decreased the Zn of the exchangeable fraction ($P < 0.05$). Schwab et al. (2008) also reported a lower concentration of exchangeable Zn in all sections of a soil column when citric acid was applied at 10 mM compared to treatments with 0.1 and 1 mM.

A significant decrease of the water-soluble Zn fraction in treatments T10 and CT5 of soil G and in all the treatments of soil C was observed relative to the untreated soil ($P < 0.05$). Conversely, the acid-soluble Zn fraction significantly increased in some treatments (C10, T10, and CT5 of soil G; T0.5, C10, and CT5 of soil C) after the experiment ($P < 0.05$). This was probably due to the increase in soil pH during the experiment or to the formation of metal–organic complexes that were weakly adsorbed to the soil particles (Barona et al., 2001).

Similar to Cu, the concentration of Zn that was bounded to Fe and Mn oxides significantly decreased after the experiment in most cases ($P < 0.05$) due to the dissolution of these minerals caused by LMWOAs and the reducing conditions in the columns. Additionally, these reducing conditions could have led to the significant increase in Zn that was bounded to the organic matter and sulfides in soil G ($P < 0.05$).

At the end of the experiment, there were no significant differences in the sums of all of the fractions of Zn between the untreated soils and the soils. Neither the citric acid nor the tartaric acid was able to effectively mobilize a significant amount of Zn in either soil.

In spite of the fast degradation of these LMWOAs, previous studies (Evangelou et al., 2006; Nascimento et al., 2006) have reported that the application of citric acid at high doses in pot experiments is able to enhance Cu and Cd uptake by plants in comparison to other LMWOAs (oxalic, vanillic and tartaric acids).

4. Conclusions

The chemical speciation and mobility of metals in old contaminated mine soils was significantly affected by the presence of LMWOAs. The results of the one-step extraction experiment showed that the desorption behavior of metals was related to the types of organic acid that were applied and their concentrations. The addition of higher concentrations of organic acids significantly increased metal desorption. Citric acid demonstrated a higher metal mobilization ability than tartaric acid. Metal desorption was mainly determined by the decrease of solution pH and the dissolution of Fe and Mn oxides by these LMWOAs, in contrast to the formation of soluble metal–organic complexes predicted by the speciation modeling using Visual Minteq.

The results of the soil column study showed that low concentrations of organic acids (0–0.5 mM) did not cause substantial effects on metal desorption. Moreover, none of the treatments with LMWOAs was able to mobilize a significant amount of Zn in soils. However, high concentrations of citric acid (5–10 mM) remarkably promoted Cu mobilization and reduced the amount of Cu that was retained in the soil, especially from the exchangeable and the Fe and Mn oxide fractions. Despite the inefficiencies of LMWOAs that have been reported in previous works, high doses of citric acid appeared to be an effective Cu extracting agent for these mine soils. This compound, used as an alternative to synthetic chelating agents, could facilitate assisted phytoextraction techniques with minimal environmental risk.

Acknowledgments

The authors are grateful to Juan Candela and Maria José Arboledas for their assistance on soil analyses at the laboratory of the Department of Edaphology of the Polytechnic University of Madrid (Spain). This work was financed by the Spanish Ministry of Science and Innovation (Project CTM2009-13140-C02-01).

References

Barona, A., Aranguiz, I., Elías, A., 2001. Metal associations in soil before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environ. Pollut. 113, 79–85.

Barton, L., Abadía, J., 2006. Iron Nutrition in Plants and Rhizospheric Microorganisms. Springer, Dordrecht.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol. 31, 860–865.

Charlatchka, R., Cambier, P., 2000. Influence of reducing conditions on solubility of trace metals in contaminated soils. Water Air Soil Pollut. 118, 143–167.

Council of the European Communities, 1986. Council directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. Official J. Eur. Commun. L181, 6–12.

Day, P.R., 1965. Particle fractionation and particle-size analysis. In: Black, C.A. (Ed.), Methods of Soil Analysis, Part 1. American Society of Agronomy, Madison, pp. 545–567.

Evangelou, M.W.H., Ebel, M., Schaeffer, A., 2006. Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*. Chemosphere 63, 996–1004.

Evangelou, M.W.H., Ebel, M., Schaeffer, A., 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68, 989–1003.

Evangelou, M.W.H., Ebel, M., Hommes, G., Schaeffer, A., 2008. Biodegradation: the reason for the inefficiency of small organic acids in chelant-assisted phytoextraction. Water Air Soil Pollut. 195, 177–188.

Gao, Y., He, J., Ling, W., Hu, H., Liu, F., 2003. Effects of organic acids on copper and cadmium desorption from contaminated soils. Environ. Int. 29, 613–618.

Gustafsson, J.P., 2011. Visual MINTEQ, v.3.0. Royal Institute of Technology, Department of Land and Water Resources Engineering, Stockholm. <http://www2.lwr.kth.se/English/OurSoftware/Vminteq/index.htm>.

ISO 11466, 1995. Soil quality: extraction of trace elements soluble in aqua regia. International Organization for Standardization, Geneva.

IUPAC, 1994. Stability Constants Database, Version 2.62. Academic Software Royal Society of Chemistry, Cambridge.

Johnson, S.E., Loeppert, R.H., 2006. Role of organic acids in phosphate mobilization from iron oxide. Soil Sci. Soc. Am. J. 70, 222–234.

Jones, D.L., 1998. Organic acids in the rhizosphere – a critical review. Plant Soil 205, 25–44.

Liu, D., Islam, E., Li, T., Yang, X., Jin, X., Mahmood, Q., 2008. Comparison of synthetic chelators and low molecular weight organic acids in enhancing phytoextraction of heavy metals by two ecotypes of *Sedum alfredii* Hance. J. Hazard. Mater. 153, 114–122.

Martell, A.E, Smith, R.M., 1976. Critical Stability Constants, vol. 6. Plenum Press, New York.

Nascimento, C.W.A., 2006. Organic acids effects on desorption of heavy metals from a contaminated soil. Sci. Agric. 63, 276–280.

Nascimento, C.W.A., Amarasiriwardena, D., Xing, B., 2006. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environ. Pollut. 140, 114–123.

Navarro, M.C., Pérez-Sirvent, C., Martínez-Sánchez, M.J., Vidal, J., Tovar, P.J., Bech, J., 2008. Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone. J. Geochem. Explor. 96, 183–193.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L. et al. (Eds.), Methods of Soil Analysis, Part 3. Chemical Methods. Soil Science Society of America and American Society of Agronomy, Madison, pp. 961–1010.

Pastor, J., Aparicio, A.M., Gutiérrez-Maroto, A., Hernández, A.J., 2007. Effects of two chelating agents (EDTA and DTPA) on the autochthonous vegetation of soil polluted with Cu, Zn and Cd. Sci. Total Environ. 378, 114–118.

Qin, F., Shan, X.Q., Wei, B., 2004. Effects of low-molecular-weight organic acids and residence time on desorption of Cu, Cd, and Pb from soils. Chemosphere 57, 253–263.
Rhoades, J.D., 1982. Cation exchange capacity. In: Page, A.L. et al. (Eds.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, second ed. American Society of Agronomy, Madison, pp. 149–164.
Serjeant, E.P., Dempsey, B., 1979. Ionisation Constants of Organic Acids in Aqueous Solutions. Pergamon, Oxford.
Schwab, A.P., Zhu, D.S., Banks, M.K., 2008. Influence of organic acids on the transport of heavy metals in soil. Chemosphere 72, 986–994.
Shuman, L.M., 1982. Separating soil iron- and manganese-oxide fractions for microelement analysis. Soil Sci. Soc. Am. J. 46, 1099–1102.
Smith, B.F.L., Mitchell, B.D., 1987. Characterization of poorly ordered minerals by selective chemical methods. In: Wilson, M.J. (Ed.), A Handbook of Determinative Methods in Clay Mineralogy. Blackie & Son Ltd, Glasgow, pp. 275–294.
Smith, R.M., Martell, A.E., Motekaitis, R.J., 2001. NIST crititically selected stability constants of metal complexes database. NIST Standard Reference Database 46, Version 6.0. National Institute of Standard and Technology, Gaithersburg.
Smith, R.M., Martell, A.E. and Motekaitis, R.J. 2003. NIST crititically selected stability constants of metal complexes database. NIST Standard Reference Database 46, Version 7.0. National Institute of Standard and Technology, Gaithersburg.
Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.
Vamerali, T., Bandiera, M., Mosca, G., 2010. Field crops for phytoremediation of metal-contaminated land. A review. Environ. Chem. Lett. 8, 1–17.
Vesely, T., Tlustos, P., Szakova, J., 2012. Organic acid enhanced soil risk element (Cd, Pb and Zn) leaching and secondary bioconcentration in water lettuce (*Pistia stratiotes* L.) in the Rhizofiltration process. Int. J. Phytoremediation 14, 335–349.
Zelazny, L.W., He, L., Vanwormhoudt, A., 1996. Charge analysis of soils and anion exchange. In: Sparks, D.L. et al. (Eds.), Methods of Soil Analysis, Part 3: Chemical Methods. Soil Science Society of America and American Society of Agronomy, Madison, pp. 1231–1253.